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Original Article

Comparison of Total Phenolic Content and Antioxidant Properties of Six Species of *Ocimum*

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ABSTRACT

The current study is being conducted and focused to evaluate the phenolic content and antioxidative properties of six selected *Ocimum* species (*Ocimum basilicum* L., *Ocimum kilimandscharicum* Guerke, *Ocimum gratissimum* L., *Ocimum canum* Sims., *Ocimum tenuiflorum* L. (green type) and *Ocimum tenuiflorum* L. (Purple type) which is being investigated. Leaves from each type of plant are taken and extracted using methanol. Total phenolic content was estimated using Folin-Ciocalteu reagent and antioxidant activity was assessed using iron (III) reduction, carotene-linoleic acid bleaching, 1,1-diphenyl-2-picrylhydrazyl and superoxide anion free radical scavenging assays. The outcome of the present investigation demonstrates that the six species of *Ocimum* where predominant in phenolics and flavonoid content, the chemical nature of the phenolics and flavonoids, in alone or acting synergistically, facilitates them to act as reducing agents and free radical scavengers. Consumption of these kinds of extracts in the form of preservatives in the food industry may lead to benefits in human health too, mainly in protection against excessive oxidative stress. The findings of the present study exhibited varying scavenging properties against different free radicals.

Keywords: *Ocimum* sp., phenolic content, Free radical, antioxidant.

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INTRODUCTION

Chemical constituents from plants having high concentration of antioxidant properties are observed to play an important role in prevention of degenerative diseases.[1] Since ages spices and herbs are used to add flavour in foods and beverages, which can also serve the purpose of preservatives. Plant species which are used for preservation purposes are mainly due to anti oxidative properties. Supplementing human diet with compounds deactivating free radicals may have beneficial effects on health.[2] Phenolic compounds are found to be involved in cell defense against free radicals.[3] Plants belonging to Lamiaceae family have phenolic compounds which are high in antioxidant activity.[4] Rise in interest among manufacturers from food industry and also consumers towards foods with beneficial health effects can be observed.[5]

Phenolic compounds comprise a major group of plant secondary metabolites. They are biochemically synthesized via the shikimate pathway, which produces the group of phenolics called phenylpropanoids.[6] They can act as antioxidants by donating hydrogen to highly reactive radicals, thereby preventing further radical formation.[7] It has been reported that phenolic compounds of plant materials shown to neutralize free radicals in various model systems.[8] Free radicals attack the unsaturated fatty acids in the biomembranes resulting in membrane fluidity.[9] For these reasons antioxidants are of interest for the treatment of many kinds of cellular degeneration.[10] Increasing experimental evidence has suggested that diets rich in phenolic compounds are associated with a longer life expectancy[11] and have also been found to exhibit many health-related properties because of their antioxidant activities. Polyphenols, including vitamins, pigments and flavonoids, possess antimutagenic properties as well as blood glucose decreasing activity.[12] A great number of plants worldwide showed a strong antioxidant activity[13] and a powerful scavenger activity against free redicals.[14]

The plants of the genus *Ocimum* (Lamiaceae) comprises more than 30 species are widely distributed in the tropical and subtropical regions of Asia, Africa and Central and South America[15] and have long been extensively used in folk medicine in the India and most other countries for thousands of years in the treatment of various ailments including rheumatism, paralysis, epilepsy, high fever, diarrhea, sunstroke, influenza, gonorrhea, mental illness, abdominal pains, skin diseases, colds, coughs, measles, antipyretic, antihelmentic, stomatic, anti-emetic, antimalarial, antiviral, anti-inflammatory.[16,17] It is also a source

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of aroma compounds and essential oils containing biological active constituents that possess antibacterial,[18-22] antifungal,[23,24] insecticidal,[25] nematicidal,[26] antidiabetic,[17] and fungistatic properties.[27] The active compounds present as volatile oil from the leaves consist mainly of eugenol, thymol, citrol, geraniol, camphor, linalool l and methyl cinnamate.[28-33] Recently, we are reported that methanolic leaf extract of *Ocimum basilicum* L. possesses anti-hematotoxicity activity against benzene induced in mice.[34]

The following six species are very common in West Bengal, India and therefore have been chosen for the present investigation, namely, *Ocimum basilicum* L., *Ocimum gratissimum* L., *Ocimum kilimandscharicum* Guerke, *Ocimum canum* Sims., *Ocimum tenuiflorum* L. (green type), *Ocimum tenuiflorum* L. (purple type). Several studies on *Ocimum* phenolic compounds and their antioxidant activities have been reported,[35] and various aqueous solutions of acetone, methanol, and ethanol have also been used to extract the free phenolic compounds from *Ocimum*.[36-40] However, it is difficult to compare data within the literature, owing to the different antioxidant activity evaluation methods and extraction solvents used by various researchers. Moreover, antioxidant compounds present in *Ocimum* extracts are complex, and their activities and mechanisms would largely depend on the composition and conditions of the test system. Many authors had stressed the need to perform more than one type of antioxidant activity measurement to evaluate the antioxidant activity of plant. In this study, DPPH radical scavenging activity, SO radical scavenging activity, NO radical scavenging activity, Total phenolic content, Total flavonoid content, and Total antioxidant activities were evaluated of methanol solvent extract prepared from *Ocimum*.

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteau's (FC) reagent, Aluminum Chloride ($AlCl_3$), Ammonium Molybdate, Ascorbic Acid, Ethylenediaminetetraacetic Acid (EDTA), Ferric Chloride ($FeCl_3$), Ferrous Sulphate ($FeSO_4$,$7H_2O$), potassium acetate, potassium ferricyanide, potassium persulphate, riboflavin, Sodium Nitrite ($NaNO_2$) and sulfuric acid were obtained from Merck, India. DPPH, ABTS, gallic acid, quercetin, Nitro Blue Tetrazolium (NBT), tannic acid and 2, 4, 6-tripyridyl-s-triazine (TPTZ) were purchased from Sigma-Aldrich, (St. Louis, MO, USA). All other reagents were of analytical grade.

Plant Material

Fresh leaves of *Ocimum basilicum* L., *Ocimum kilimandscharicum* Guerke, *Ocimum gratissimum* L., *Ocimum canum* Sims., *Ocimum tenuiflorum* L. (green type) and *Ocimum tenuiflorum* L. (Purple type) (Figure 1) were collected from medicinal and aromatic plant garden, Department of Botany, University of Kalyani, Kalyani, West Bengal, India, in August of 2011 (Table 1), which is located at $22^0 57'$ N latitude, $88^0 22'$ E longitude with an average altitude of 9.75 m above mean sea level. The taxonomic identification of plant material was confirmed by Dr. G. G. Maity, Professor of Taxonomist, Taxonomy and Plant systematic Unit, Department of Botany, University of Kalyani. The voucher specimens (143, 148, 149, 150, 163 and 169 KUH respectively) was deposited and preserved in the Department of Botany, University of Kalyani, Kalyani, and West Bengal, India, for reference.

Preparation of methanolic plant extract

Each plant material was dried in the shade at room temperature and ground in a grinder with a 2 mm diameter mesh. The dried and powdered leaves (20 g) were successively with 100 mL of methanol (1:5 w/v) by using a Soxhlet extractor for 72 hr at a temperature not exceeding the boiling point of the solvent.[41] The extracts were filtered using Whatman filter paper (No. 1) and then concentrated in vacuo at 40°C using a rotary evaporator (Buchi Rotavapor R-200). The extracts were then lyophilized and kept in the dark at + 4°C until tested.

Determination of total phenolic content

Total Phenol Contents (TPC) were determined using Folin-ciocalteu reagent and expressed as Gallic Acid Equivalents (GAE).[42] Briefly, the extracts (0.5 mL; 1 mg/mL stock solution) were mixed with 0.5 mL of distilled water and 1 mL of FC reagent (pre-diluted, 10 times, with distilled water) and incubated for 5 min at room temperature (27±2°C). After incubation, 2 mL of 700 mM sodium carbonate was added in the reaction mixtures, mixed and kept in dark for 45 min at room temperature. The absorbances of the samples were measured at 765 nm using a UV-vis spectrophotometer (CECIL, CE 7200; Cambridge, UK). A calibration curve was prepared using standard solutions of gallic acid ranging from 10 to 160 μg/mL (r2 =0.992). The amount of phenolics in different extracts was calculated from the calibration curve and was expressed as mg Gallic Acid Equivalent (GAE) per gram of extract obtained from the studied species.

Determination of total flavonoid

Total flavonoids content was determined by a Chang *et al.*,[43] method using quercetin as a reference compound with minor modifications. The samples (0.5 mL extract; 1 mg/mL stock) were mixed with 1.5 mL distilled water and 0.2 mL 5% $NaNO_2$ and the resultant solution could stand for 2 min at room temperature (27±2°C). Subsequently, 0.2 mL of 10% $AlCl_3$ in ethanol and 0.6 mL 1N sodium hydroxide were added successively with vortexing in each step. The samples were incubated in dark at room temperature for 10 min and the absorbance was measured at 510 nm using a spectrophotometer. The amount of total flavonoid in the samples was quantified from the calibration curve of quercetin (ranging from 10 to 200 μg/mL; r2 =0.997) and was

expressed as mg Quercetin Equivalent (QE) flavonoid per gram of *Ocimum* leaf extract.

Determination of total antioxidant activity

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.*,[44] with modifications. The assay is based on the reduction of Mo(VI)-Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid PH. 0.5 mL each extract sample (from 1 mg/mL stock solution) was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 90°C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer (CECIL, CE 7200; Cambridge, UK) against blank after cooling to room temperature. Methanol (0.3 mL) in place of extract is used as the blank. The antioxidant activity is expressed as the units of Ascorbic Acid Equivalent (AAE) using the calibration curve ranging from 10 to 300 µg/mL; r2 = 0.994 in per gram of methanolic extract.

DPPH radical scavenging activity

The DPPH radical scavenging activity was measured according to Brand Williams[45] with some modifications. An aliquot (50 µL) of extract of different concentrations (50 µg/mL to 1000 µg/mL) was added to 1.2 mL of 6×10^{-5} M DPPH solution and shaken vigorously. The tubes were then incubated in the dark at room temperature for 15 min. A DPPH control sample was prepared without any extract, and methanol was used for the baseline correction. Decreases in the absorbance at 517 nm were measured using a UV-visible spectrophotometer. Results were expressed as percentage scavenging activity of the DPPH• radical which was calculated according to the following equation:

Scavenging activity (%) = [(A control - A sample) / A control]
100

Where, $A_{control}$ and A_{sample} are the absorbance of the control and the test sample, respectively. The 50% scavenging of the DPPH radical (IC_{50}) was estimated from a graphical plot where percent scavenging (inhibition) was plotted against varying concentrations of an extract.

Superoxide (SO) scavenging activity

The SO radicals were generated by modified method based on Beauchamp and Fridovich.[46] The assay was based on the potentiality of the samples to inhibit blue formazan formation by scavenging the superoxide radical generated in riboflavin-light-NBT system.[28] The samples of different concentrations were prepared in 50 mM sodium phosphate buffer (pH 7.6). The total volume of reaction mixture was 3 mL which was prepared by sequential addition of 1 ml of sample solution, 1.8 mL of 50 mM sodium phosphate buffer pH 7.6, 20 µL 2.66 mM riboflavin, 80

Figure 1: Mother plant of *Ocimum basilicum* L., *Ocimum kilimandscharicum* Guerke, *Ocimum gratissimum* L., *Ocimum canum* Sims., *Ocimum tenuiflorum* L. (green type) and *Ocimum tenuiflorum* L. (Purple type).

Table 1: Plants and plant parts used.

Sl. No.	Scientific name	Common name	Family	Parts used
1.	*Ocimum basilicum* L.	Sweet basil	Lamiaceae	Dried leaves
2.	*Ocimum kilimandscharicum* Guerke.	Camphor basil	Lamiaceae	Dried leaves
3.	*Ocimum gratissimum* L.	Shrubby basil	Lamiaceae	Dried leaves
4.	*Ocimum canum* Sims.	Hoary Basil	Lamiaceae	Dried leaves
5.	*Ocimum tenuiflorum* L. (green type)	Holy basil	Lamiaceae	Dried leaves
6.	*Ocimum tenuiflorum* L. (purple type)	Sacred Basil	Lamiaceae	Dried leaves

μL 12 mM EDTA and 100 μL 1.22 mM NBT. The photo-induced reactions were initiated by illuminating the reaction mixtures with a 20 W luminous bulb within an aluminium lined box for 90 sec at room temperature. The non-illuminated reaction mixture was used as blank. After completion of reaction, the absorbances were measured at 590 nm. The IC_{50} values were determined from the percent SO radical scavenging and that was obtained from the formula represented in previous sections.

Assay of nitric oxide scavenging activity

The NO scavenging assay[47] was performed by mixing 1 mL Sodium Nitroprusside (SNP) solution (10 mM SNP in 20 mM sodium phosphate buffer, pH 7.4) with 1 mL extract at varying (0-1000 μg) concentrations under illumination (25 W tungsten bulbs) for 2 hr. After illumination, 2 mL of the Greiss reagent (2% sulphanilamide in 4% H_3PO_4) and 0.2% naphthylethylenediamine dihydrochloride in 1:1 ratio (v/v; prepared fresh) was added to the mixture and incubated for 10 min at RT. The absorbance was recorded at 542 nm. The percentage scavenging activity of different extract samples against NO radical were calculated using the following equation:

Scavenging activity (%) = [(A control - A sample) / A control] 100

Statistical analysis

Critical Difference (CD) at 0.05 probability level was performed to assess the significant level, if any, between and among the estimates of phenolics (TPC, TFC, and TTC) and TAA for different extraction conditions. CD at 0.05 probability level was also ascertained between/among the estimates of different antioxidant assays (DPPH, SO and NO) to assess significant variation, if any. Pearson correlation coefficient (r) was determined between the studied attributes like TPC, TFC, TTC and TAA considering extraction conditions at 47 degrees of freedom to ascertain whether there exists any interrelationship between and among them or not.

RESULTS

Total Phenolic Content (TPC)

Results exhibit that among the tested samples, maximum quantity of TPC (Figure 2) was observed in *O. tenuflorum* (P) i.e. 107.25±6.1 (mg GAE/g extract sample) followed by the TPC content of *O. basilicum, O. gratissimum, O. canum, O. kilimandscharicum* and *O. tenuflorum* (W) methanolic extract. The estimated quantity of TPC in the six *Ocimum* extracts varied between (53.33±6.0) to (107.25±6.1) mg GAE/g extract. The yield of TPC is found to vary significantly (p<0.05) among the six *Ocimum* sp. considered for the present study.

Total Flavonoid Content (TFC)

The estimated amount of TFC (mg QE/g) was recorded high in *O. basilicum* (167.26±12.4) while low TFC was estimated in *O. canum* (96.52±10.6). A significant variation in TFC was observed among all the extracts obtained from the studied *Ocimum* sp. (Figure 2).

Total Antioxidant Activity (TAA)

TAA content (mg AAE/ g) in the entire sample of *Ocimum* extracts are shown in (Figure 2). Results confirmed that highest and lowest TAA was calculated as (245.00±5.4) and (113.92±5.4) for *O. basilicum* and *O. tenuflorum* (W) extracts respectively. The TAA content of other studied species of *Ocimum* lied between them with significant (p<0.05) variation between them.

DPPH radical scavenging activity

DPPH radical scavenging activity (%) was studied in methanolic extracts of different *Ocimum* species namely *O. tenuflorum* (P), *O. basilicum, O. gratissimum, O. canum, O. kilimandscharicum* and *O. tenuflorum* (W) and compared with quercetin (a reference flavonoid) at different concentrations (100-1000 μg/ mL) and the antioxidant activity of the samples is depicted as percentage (%) scavenging activity (Figure 3a). Among the Ocimum species studied, *O. basilicum* exhibits highest DPPH scavenging activity (68.63±2.2) % at a concentration of 1000 μg/ mL whereas the lowest antioxidant activity was recorded for *O. tenuflorum* (P) which was (37.07±3.3) % at maximum concentration (1000 μg/

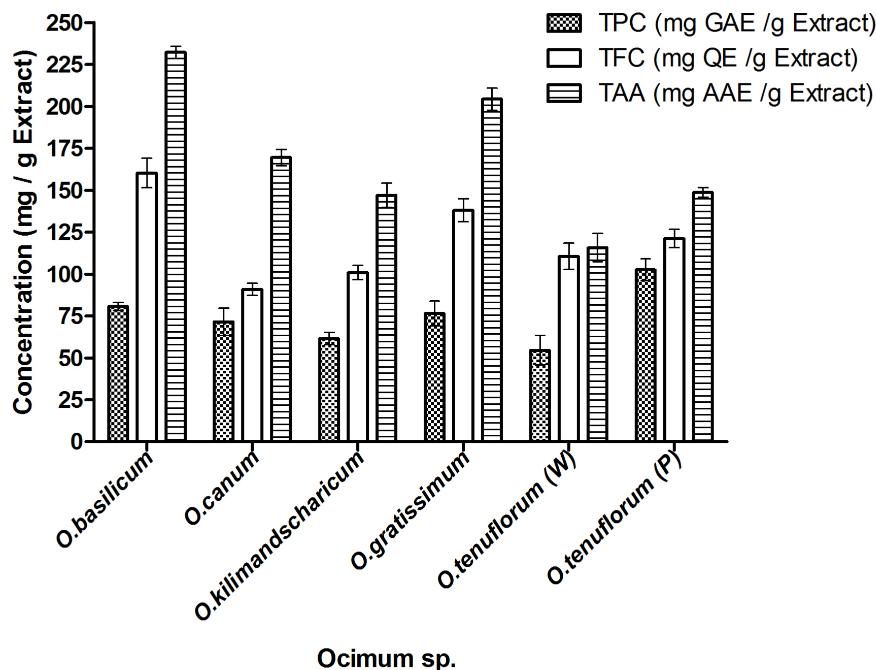

Figure 2: Phenolic yield (TPC, TFC and TTC) of six species of *Ocimum*.

mL). Antioxidant activity of all the samples was found to be dependent on the test.

The IC_{50} values (µg/ mL) of all the samples were also calculated and found between a range (378.45±21.3) for *O. basilicum* and (983.65±25.6) for *O. tenuflorum* (W) whereas the IC_{50} value of quercetin was calculated as 137.20±31.6 in quercetin. DPPH radical scavenging was found in the order as *O. basilicum* > *O. gratissimum* > *O. canum* > *O. kilimandscharicum* > *O. tenuflorum* (P) > *O. tenuflorum* (W). The lower the IC_{50} value, higher the antioxidant activity of a sample.

Superoxide (SO) scavenging activity

SO scavenging activity (%) is also studied (Figure 3b) and maximum SO scavenging activity was exhibited by *O. basilicum* (367.52±20.2) followed by *O. canum* (974.68±20.4) as indicated by their calculated IC_{50} value (µg/mL). Other samples also found to possess SO scavenging activity at moderate level with IC_{50} value greater than 1000 µg/mL. In all the sample methanolic extracts, a dose dependent enhancement in SO scavenging activity was observed. However, the SO scavenging activity of all the samples was lesser than quercetin whose IC_{50} value was observed as (305.36±17.6) µg/mL.

Assay of Nitric Oxide (NO) scavenging activity

Maximum NO scavenging activity (%) was observed (Figure 3c) in methanolic extract of *O. basilicum* with an IC_{50} value (µg/mL) of (676.09±22.0) whereas other studied samples were found to have moderate NO scavenging activity with IC_{50} values >1000 µg/mL and hence cannot be detected. Likewise, DPPH and SO scavenging activity, all the samples exhibited NO scavenging activity in a dose dependent manner including quercetin whose IC_{50} value was deduced to be (129.46±13.8) µg/mL which indicates very high NO scavenging capacity of quercetin as compared to other studied samples.

DISCUSSION

The study that was done focused on determining the methanolic extract from the shade-dried leaves of six different *Ocimum* species that had the most phenolics and flavonoids at different levels. Shade-dried leaf samples are frequently better for polyphenol investigations because they last longer, give more extract, and have more polyphenol content than fresh leaves.[48-50] Solvent extraction at higher temperatures and longer extraction times makes it easier for phytoconstituents to leave the extraction solvents.[33] The release of secondary metabolites and the antioxidant properties of plants change. These changes happen not only between plants of the same species but also between different species. We can also see that it varies amongst individuals of the same species, which could be because of how the samples were treated or because of differences in the environment. Many studies on different plant species[50-52] show that methanol is the best solvent for extracting low molecular weight phytoconstituents, such as phenolics and flavonoids, that are polar or semi-polar. Ahmed et al.[53] employed ethanol to extract phytoconstituents from *O. basilicum* L. leaves and stems, which shows that solvents other than methanol can also be used to extract phytoconstituents. Among the six *Ocimum* species studied, the methanolic extract of *O. basilicum* L. leaves had a high flavonoid content and total antioxidant activity. This suggests that the flavonoids may be one of the main groups that

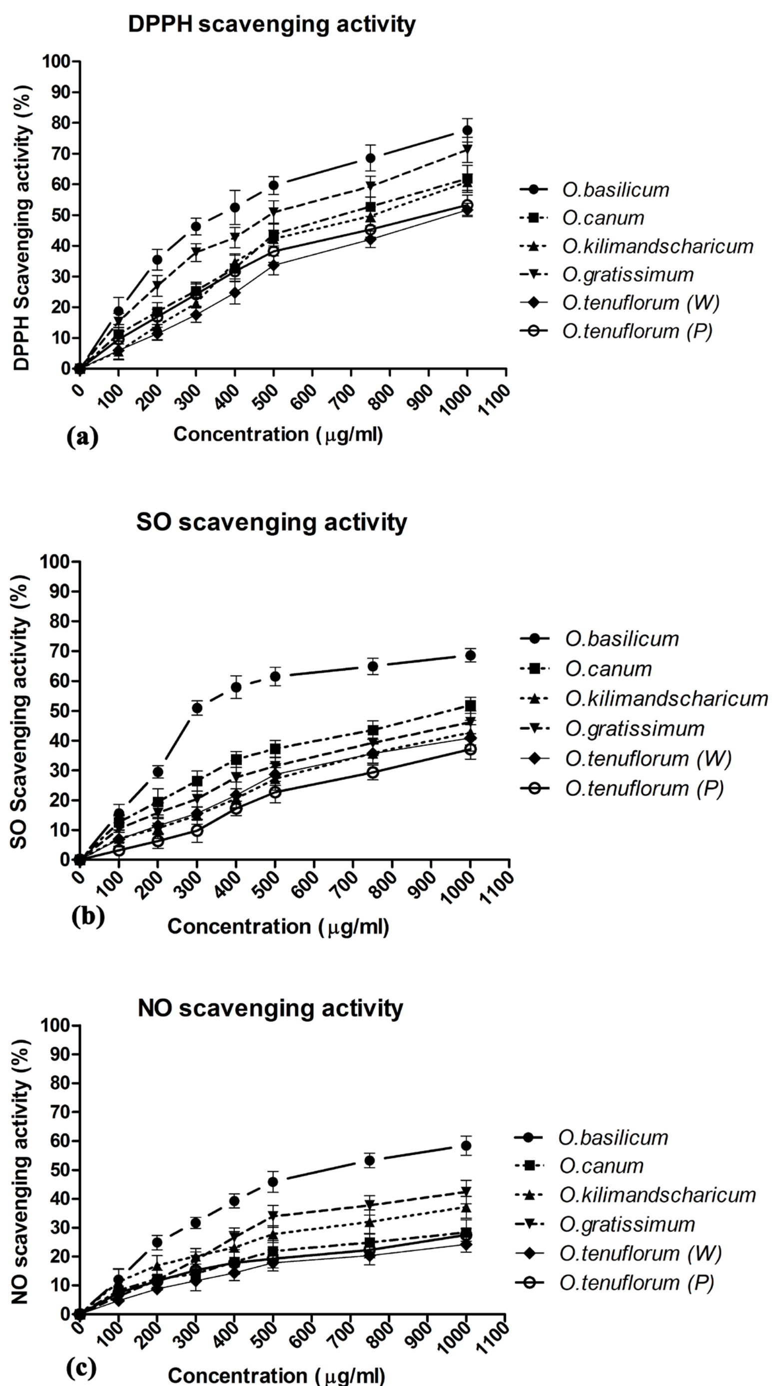

Figure 3: (a) Total Antioxidant Activity (TAA), (b) SO scavenging activities, and (c) NO scavenging activities of six species of *Ocimum*.

contribute to the antioxidant property of the samples studied. These results are consistent with some previous studies[36,50,53,54] that found a strong link between phenolics, and flavonoids and the antioxidant property of the plant species studied. The chemical structure of phenolics and flavonoids, whether they work alone or together, makes them good at reducing agents and scavenging free radicals.[55] Using these kinds of extracts as food preservatives may also be good for people's health, especially when it comes to protecting them from too much oxidative stress. The results of this study showed that the *Ocimum* species tested have distinct scavenging abilities against different free radicals, such as DPPH, nitric oxide, and superoxide. This suggests that the antioxidant capacity of these species is related to the high levels of polyphenols in their leaves.

CONCLUSION

From our study we conclude that the six *Ocimum* species have varying degrees of phenolic and flavonoid content. The chemical makeup of flavonoids and phenolics allows them to function as scavengers of free radicals and reducing agents, either alone or in combination. These extracts are used in the food industry as preservatives, and their consumption may also help human health, primarily by protecting against excessive oxidative stress. Different scavenging properties against various free radicals were demonstrated by the investigation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TPC: Total phenolic content; **TFC:** Total flavonoid content; **TTC:** Total tannin content; **TAA:** Total antioxidant activity; **FL:** Fresh leaves; **SDL:** Shade dried leaves; **DPPH:** Diphenyl-1-picrylhydrazyl; **SO:** Superoxide; **FRAP:** Ferric reducing antioxidant power; **NBT:** Nitro blue tetrazolium; **TPTZ:** 2, 4, 6-tripyridyl-s-triazine; **CD:** Critical difference.

REFERENCES

1. Velioglu, Y.; Mazza, G.; Gao, L.; Oomah, B.D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem. 1998; 46(10): 4113-7.
2. Lutomski, J. Znaczenie ziol w terapii i dietetyce. Herba Pol. 2002; 48(4): 300-10.
3. Szeto, Y.T.; Tomlinson, B.; Benzie, I.F. Total antioxidant and ascorbic acid content of fresh fruits and vegetables: implications for dietary planning and food preservation. Br J Nutr. 2002; 87(1): 55-9.
4. Song, H.; Wang, Y.S.; Sun, C.C.; Wang, Y.T.; Peng, Y.L.; Cheng, H. Effects of pyrene on antioxidant systems and lipid peroxidation level in mangrove plants, *Bruguiera gymnorrhiza*. Ecotoxicology. 2012; 21(6): 1625-32.
5. Özkan, G.; Sağdiç, O.; Özcan, M. Inhibition of pathogenic bacteria by essential oils at different concentrations. Food Sci. Technol. Int. 2003; 9(2): 85-8.
6. Singer, A.C.; Crowley, D.E.; Thompson, I.P. Secondary plant metabolites in phytoremediation and biotransformation. Trends Biotechnol. 2003; 21(3): 123-30.
7. Lapornik, B.; Prošek, M.; Wondra, A.G. Comparison of extracts prepared from plant by-products using different solvents and extraction time. J. Food Eng. 2005; 71(2): 214-22.
8. Zhang, J.; Kirkham, M.B. Antioxidant responses to drought in sunflower and sorghum seedlings. New Phytol. 1996; 132(3): 361-73.
9. Dean, R.T.; Gieseg, S.; Davies, M.J. Reactive species and their accumulation on radical-damaged proteins. Trends Biochem. Sci. 1993; 18(11): 437-41.
10. Behera, B.C.; Verma, N.; Sonone, A.; Makhija, U. Determination of antioxidative potential of lichen Usnea ghattensis *in vitro*. LWT- Food Sci. Technol. 2006; 39(1): 80-5.
11. Hertog, M.G.; Kromhout, D.; Aravanis, C.; Blackburn, H.; Buzina, R.; Fidanza, F.; Katan, M.B. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med. 1995; 155(4): 381-6.
12. Thompson, L.U.; Yoon, J.H.; Jenkins, D.J.A.; Wolever, T.M.S.; Jenkins, A.L. Relationship between polyphenol intake and blood glucose response of normal and diabetic individuals. Am J Clin Nutr. 1984; 39(5): 745-1.
13. Katalinic, V.; Milos, M.; Kulisic, T.; Jukic, M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food chem. 2006; 94(4): 550-7.
14. Kumaran, A.; Karunakaran, R.J. Activity-guided isolation and identification of free radical-scavenging components from an aqueous extract of *Coleus aromaticus*. Food Chem. 2007; 100(1): 356-61.
15. Paton, A.J. A synopsis of *Ocimum* L. (Labiatae) in Africa. Kew Bull. 1992; 47(3): 403-35.
16. Bhattacharjya D, Adhikari S, Bhuimali A, Ghosh PD, Saha S. *Ocimum* phytochemicals and their potential impact on human health. In: Rao V, Mans D, Rao L, editors. Phytochemicals in Human Health. IntechOpen, UK; 1999. (2019).
17. Sharma, A.D.; Kaur, I.; Angish, S.; Thakur, A.; Sania, S.; Singh, A. Comparative phytochemistry, antioxidant, antidiabetic, and anti-inflammatory activities of traditionally used *Ocimum basilicum* L. *Ocimum gratissimum* L.,and *Ocimum tenuiflorum* L. Biotechnol. 2022; 103(2): 131-42.
18. Prasad, G.; Abhay Kumar, A.K.; Singh, A.K.; Bhattacharya, A.K.; Kamla Singh, K.S.; Sharma, V.D. Antimicrobial activity of essential oils of some *Ocimum* species and clove oil. Fitoterapia. 1986; 57(6): 429-32.
19. Nakamura, C.V.; Ueda-Nakamura, T.; Bando, E.; Melo, A.F.; Cortez, D.A.; Dias Filho, B.P. Antibacterial activity of *Ocimum gratissimum* L. essential Oil. Mem Inst Oswaldo Cruz. 1999; 94(5): 675-8.
20. Adiguzel, A.; Gulluce, M.; Sengul, M.; Ogutcu, H.; Şahin, F.; Karaman, I. Antimicrobial effects of *Ocimum basilicum* (Labiatae) extract. Turk J Biol. 2005; 29(3): 155-60.
21. Verma, R.S.; Bisht, P.S.; Padalia, R.C.; Saikia, D.; Chauhan, A. Chemical composition and antibacterial activity of essential oil from two *Ocimum* spp. grown in sub-tropical India during spring-summer cropping season. J. Tradit. Med. 2011; 6(5): 211-7.
22. Saha, S.; Dhar, T.N.; Sengupta, C.; Ghosh, P.D. Biological Activities of Essential Oils and Methanol Extracts of Five *Ocimum* Species against Pathogenic Bacteria. Czech J. Food Sci. 2013; 31(2): 194-202.
23. Lemos, J.D.A.; Passos, X.S.; Fernandes, O.D.F.L.; Paula, J.R.D.; Ferri, P.H.; Souza, L.K.H.; Silva, M.D.R.R. Antifungal activity from *Ocimum gratissimum* L. towards *Cryptococcus neoformans*. Mem Inst Oswaldo Cruz. 2005; 100(1): 55-8.
24. Oxenham, S.K.; Svoboda, K.P.; Walters, D.R. Antifungal activity of the essential oil of basil (*Ocimum basilicum*). J Phytopatol. 2005; 153(3): 174-80.
25. Deshpande, R.S.; Tipnis, H.P. Insecticidal activity of *Ocimum basilicum* L. Pesticides. 1977; 11: 1e12.
26. Chatterjee, A.; Sukul, N.C.; Laskar, S.A.N.D.; Ghoshmajumdar, S. Nematicidal principles from two species of Lamiaceae. J. Nematol. 1982; 14(1): 118-20.
27. Reuveni, A.; Fleischer, R.; Putievsky, E. Fungistatic activity of essential oils from *Ocimum basilicum* chemotypes. J. Phytopatol. 1984; 110(1): 20-2.
28. Charles, D.J.; Simon, J.E. Essential oil constituents of *Ocimum kilimandscharicum* Guerke. J. Essent. Oil Res. 1992; 4(2): 125-8.
29. Mondello, L.; Zappia, G.; Cotroneo, A.; Bonaccorsi, I.; Chowdhury, J.U.; Yusuf, M.; Dugo, G. Studies on the essential oil-bearing plants of Bangladesh. Part VIII.† Composition of some Ocimum oils O. basilicum L. var. purpurascens; O. sanctum L. green; O. sanctum L. purple; O. americanum L., citral type; O. americanum L., camphor type. Flavour Fragr. J. 2002; 17(5): 335-40.
30. Viña, A.; Murillo, E. Essential oil composition from twelve varieties of basil (*Ocimum* spp.) grown in Colombia. J. Braz. Chem. Soc. 2003; 14(5): 744-9.
31. Padalia, R.C.; Verma, R.S. Comparative volatile oil composition of four *Ocimum* species from northern India. Nat. Prod. Res. 2011; 25(6): 569-75.
32. Singh, S.K.; Anand, A.; Verma, S.K.; Siddiqui, M.A.; Mathur, A.; Saklani, S. Analysis of phytochemical and antioxidant potential of *Ocimum kilimandscharicum* Linn. Int. J. Curr. Pharm. Res. 2011; 3(2): 40-6.
33. Kumari, M.; Prasad, A.; Laiq-Ur-Rahman; Mathur, A.K.; Mathur, A. Strategies for Conservation and Production of Bioactive Phytoconstituents in Commercially Important *Ocimum* Species: A Review. In: Mishra, M.K.; Kumari, N. (eds) Plants for Immunity and Conservation Strategies. 2023. Springer, Singapore. https://doi.org/10.1007/978-981-99-2824-8_4
34. Saha, S.; Mukhopadhyay, M.K.; Ghosh, P.D.; Nath, D. Effect of Methanolic Leaf Extract of *Ocimum basilicum* L. on Benzene-Induced Hematotoxicity in Mice. Evid. -Based Complement. Altern. Med. 2012; 2012(3): 176385.
35. Jyotshna Srivastava, N.; Yadav, A.K.; Shanker, K.; Gupta, M.M.; Lal, R.K. Impact of postharvest processes on major phenolic constituents and antioxidant potentials of different *Ocimum* species. J Appl Res Med Aromat Plants. 2018; 10: 9-15.
36. Javanmardi, J.; Stushnoff, C.; Locke, E.; Vivanco, J.M. Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. Food chem. 2003; 83(4): 547-50.
37. Akinmoladun, A.C.; Ibukun, E.O.; Afor, E.; Obuotor, E.M.; Farombi, E.O. Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. Sci. Res. Essays. 2007; 2(5): 163-6.
38. Gülçin, I.; Elmastaş, M.; Aboul-Enein, H.Y. Determination of antioxidant and radical scavenging activity of Basil (*Ocimum basilicum* L. Family Lamiaceae) assayed by different methodologies. Phytother. Res. 2007; 21(4): 354-61.
39. Nguyen, P.M.; Niemeyer, E.D. Effects of nitrogen fertilization on the phenolic composition and antioxidant properties of basil (*Ocimum basilicum* L.). J. Agric. Food Chem. 2008; 56(18): 8685-91.
40. Hlatshwayo, S.; Thembane, N.; Krishna, S.B.N.; Gqaleni, N.; Ngcobo, M. Extraction and Processing of Bioactive Phytoconstituents from Widely Used South African Medicinal Plants for the Preparation of Effective Traditional Herbal Medicine Products: A Narrative Review. Plants. 2025; 14: 206.
41. Lin, J.; Opoku, A.R.; Geheeb-Keller, M.; Hutchings, A.D.; Terblanche, S.E.; Jäger, A.K.; Van Staden, J. Preliminary screening of some traditional zulu medicinal plants for

anti-inflammatory and anti-microbial activities. J. Ethnopharmacol. 1999; 68(1-3): 267-74.

42. Ainsworth, E.A.; Gillespie, K.M. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat. Protoc. 2007; 2(4): 875-7.
43. Chang, C.C.; Yang, M.H.; Wen, H.M.; Chern, J.C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 2002; 10(3): 178-82
44. Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphor molybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 1999; 269(2): 337-41.
45. Brand-Williams, W.; Cuvelier, M.E.; Berset, C.L.W.T. Use of a free radical method to evaluate antioxidant activity. LWT- Food Sci. Technol. 1995; 28(1): 25-30.
46. Beauchamp, C.; Fridovich, I. Improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 1971; 44(1): 276-87.
47. Marcocci, L.; Maguire, J.J.; Droylefaix, M.T.; Packer, L. The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGb 761. Biochemical and biophysical research communications. 1994; 201(2): 748-55.
48. Rocha, R.P.; Melo, E.C.; Radünz, L.L. Influence of drying process on the quality of medicinal plants: A review. J. Med. Plants Res. 2011; 5(33): 7076-84.
49. Azwanida, N.N. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med. & Arom. 2015; 4(196): 2167-412.
50. Ojha, S.; Raj, A.; Roy, A.; Roy, S. Extraction of total phenolics, flavonoids and tannins from *Paederia foetida* L. Leaves and their relation with antioxidant activity. Pharmacog J. 2018; 10(3): 541-7.
51. Widyawati, P.S.; Budianta, T.D.W.; Kusuma, F.A.; Wijaya, E.L. Difference of solvent polarity to phytochemical content and antioxidant activity of *Pluchea indicia* less leaves extracts. Int. J. Pharm. Phyto. Res. 2014; 6(4): 850-5.
52. Pan, Y.; Deng, Z.Y.; Zheng, S.L.; Chen, X.; Zhang, B.; Li, H. Daily dietary antioxidant interactions are due to not only the quantity but also the ratios of hydrophilic and lipophilic phytochemicals. J. Agric. Food Chem. 2018; 66(34): 9107-20.
53. Ahmed, A.F.; Shi, M.; Liu, C.; Kang, W. Comparative analysis of antioxidant activities of essential oils and extracts of fennel (*Foeniculum vulgare* Mill.) seeds from Egypt and China. Food Science and Human Wellness. 2019; 8(1): 67-72.
54. Benabdallah, A.; Rahmoune, C.; Boumendjel, M.; Aissi, O.; Messaoud, C. Total phenolic content and antioxidant activity of six wild *Mentha* species (Lamiaceae) from northeast of Algeria. Asian Pac. J. Trop. Biomed. 2016; 6(9): 760-6.
55. Dai, J.; Mumper, R.J. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010; 15(10): 7313-52.

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